5-1971

Effects of DMSO and Propham on Mitosis in Root Tips of Secale cereale

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EFFECTS OF DMSO AND PROPHAM ON MITOSIS IN ROOT TIPS OF *SECALE CEREALE*

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Botany

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UTAH STATE UNIVERSITY
Logan, Utah

1971

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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in Root Tips of Secale cereale

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DMSO (dimethyl sulfoxide) and propham (isopropyl carbanilate) were investigated to determine their effect singly and in combination on mitoses in rye (Secale cereale) root tip cells. Root tips were prepared for observation by the paraffin and aceto-carmine squash methods. Results showed DMSO caused the following abnormalities: arrested metaphases, multipolar spindles, multinucleate cells, stickiness, shortened chromosomes, and mitotic inhibition. Cells from propham treated root tips exhibited the same abnormalities as those treated with DMSO. In addition, propham treatments resulted in polyploid cells and chromosome granulation. In combination, DMSO and propham caused all the abnormalities mentioned above except polyploidy. In the combination abnormalities were more frequent and occurred more rapidly.

(38 pages)

INTRODUCTION

Dimethyl sulfoxide (DMSO) is an unusually versatile material. It is a powerful solvent (10), a penetrating agent (34), and has demonstrated radio protective (5), and cryoprotective properties (22). DMSO has been extensively used in medicine and agriculture. Medically, DMSO has been used to carry drugs through the skin (21), in treatment of arthritis (29), and treatment of post amputation pain (45). Agriculturally, DMSO has been used as a carrier of minerals (26), fungicides (7), bactericides (24, 44), and herbicides (20, 23, 32, 44).

Because of DMSO's wide application to both plants and animals it seems desirable that a study be made to investigate its effects on chromosomes. A portion of the present study is designed to investigate the action of DMSO on rye root tip mitosis.

Isopropyl carbanilate (propham) is a herbicide which causes the following mitotic abnormalities: arested metaphases, polyploidy, stickiness, shortened chromosomes, multipolar anaphases, multinucleate cells, and chromosome deterioration (13, 15, 16). Propham is used primarily in controlling monocots by soil application before germination. As a herbicide, propham has three major disadvantages: 1. moisture inactivates it; 2. its action is greatly inhibited by the presence of organic matter in the soil; and 3. it is not active throughout the entire growing season.

Ennis (14) suggested that a wetting agent may increase the efficiency of propham. By combining propham with DMSO, a wetting and penetrating agent, and observing rye root tip chromosomes and primary tissue we will be

able to determine if the combinations show a detrimental additive effect and if so, to what extent.

REVIEW OF LITERATURE

DMSO

DMSO was first prepared by Alexander Saylzeff in 1867 (49) and is presently obtained from lignin, a waste product in the manufacture of paper (31). DMSO was extensively used in the early 1960's as a penetrant and became known medically as a "wonder drug" (31). A few of its medical applications included rapid healing of burns (4), treatment of mental patients (37), and as a treatment for leprosy (50). Agriculturally, DMSO has demonstrated potential as a carrier of minerals (26), fungicides (7), bactericides (24, 44), and herbicides (20, 23, 32, 44).

Labeled DMSO (18) is readily absorbed and translocated in both xylem and phloem. DMSO penetrates rapidly and can carry large molecules into plant cells and tissues; thus it has become a useful solvent for increasing pesticide penetration and, in some instances, the effectiveness of the pesticide (20, 23).

Lapham (25) reported that weeds were more effectively controlled by adding 2, 0.5, and 0.25% DMSO with the following herbicides: (2,4-dichlorophenoxy)acetic acid (2,4-D); monosodium methanearsonate (MSMA); disodium methanearsonate (DMSA); 3 amino-s-triazole (amitrole); and a 1:4 mixture of 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2-4-dichlorophenoxy)acetic acid (2,4-D). Concentrations of 2.0 and 0.5% DMSO were equally effective in enhancing herbicidal action. Hull (20) found higher concentrations of DMSO (50 and 100%) enhanced the herbicidal activity when mixed with (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) and 3, 6-dichloro-o-anisic acid (dicamba). When 20% DMSO was mixed with selective

herbicides, an antagonistic action was noted in all herbicides tested so that treated plants were barely discernible from untreated ones.

Bayer and Drever (6) reported that the effectiveness of 2,2-dichloropropionic acid (dalapon) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) was not increased when mixed with 10% and pure DMSO and sprayed on seedling oats.

DMSO alone inhibited root and shoot growth in purple nutsedge (Cyperus rotundus L.) following soil applications of 10 to 240 gallons per acre (3).

DMSO also has potential as a carrier of trace elements. Malo (27) working with avocados and Leonard (26) with citrus, reported rapid recovery of iron deficient plants after foliar application of iron solutions containing DMSO. The presence of DMSO in plants increased root uptake of phosphorus (18), magnesium, sulfur (8), calcium (17), zinc (41), and decreased uptake of sodium, rubidium (41), and nitrogen (8). Beauchamp and Crete (8), working with beans, found DMSO increased soil acidity while the availability of nitrogen and sulfur in the soil was considerably decreased. The nitrogen deficiency was due in part to the prevention of nodulation by DMSO.

The transport of large molecules across biological membranes could cause damage to the membranes. Blumberg and Ilagan (9), working with cellulose acetate membranes, reported membrane disintegration at high concentrations of DMSO. However, Dickinson et al. (12) report no significant changes in membrane structure after application of 5 and 15% DMSO. Work by Mussell et al. (33) supports these findings and suggests that the effects of DMSO on solute penetration are due to the physical characteristics of DMSO and not to the physical aspects of the membrane.

DMSO applied at low concentrations has a stimulating effect on plant growth (19). Sciuchetti and Hutchison (42) noted an 11% increase in plant height but no effect on dry weight. No significant decrease occurred in root dry weight. Nethery and Hurtt (35), working with beans, noted a decrease in plant height with DMSO concentrations as low as 1.4×10^{-3}M and a significant reduction in fresh and dry weights of roots and shoots with DMSO concentrations above 2.8×10^{-2}M.

Mussell et al. (32) reported that application of DMSO to some seeds reduced germination time and increased germination rate. Our preliminary work indicated that the chromosomes of pollen mother cells would be "sticky" if the seeds which gave rise to the test plants had been treated with DMSO. When DMSO was applied to the stems and leaves of plants from untreated seeds no stickiness was present but occasionally microspores were found containing 14 chromosomes.

DMSO is known to alter plant metabolism and enzyme systems. Raymundo et al. (38), working with tomatoes, found that DMSO inhibited production of acyclic carotenoids by inhibiting some phase of the enzyme system. Chung and Simon (11) reported that DMSO increased slightly RNase and beta-galactosidase activity and markedly decreased the activity of glycerol dehydrogenase. They suggested the effects were due primarily to DMSO's ability to alter enzyme reaction rates. Blumberg and Ilagan (9) reported DMSO inhibited glucose transport and suggested that this was caused by the viscosity of DMSO and its adverse effects on membranes. DMSO also denatures DNA and both single and double stranded RNA (47); thus, it may seriously alter plant metabolism. Keil (23) reported that inherently nontoxic compounds could, after DMSO induced absorption, alter plant metabolism to such an extent that new biologically active substances would be produced.

PROPHAM

The effect of propham on plants was first demonstrated by Templeman and Sexton (48) in 1945; they noted that low concentrations of propham inhibited cereal seedling growth but did not effect the growth of mangolds, sugar beets, flax, rape, or yellow cherlock.

Allard et al. (2) reported propham stimulated growth of tomatoes but caused stunting in potatoes, an effect which was subsequently outgrown.

Ennis (15), working with propham on crop plants, tested 13 monocots all of which were severely affected. Fifteen of 39 dicots showed a response at germination, but in only nine cases was the damage permanent.

Ennis (14) found propham was much more effective when applied to the soil than when applied to foliage.

Mitchell and Marth (30) noted propham was most effective on germinating seedlings. Application of the herbicide to older plants caused stunting and failure of seed production.

Although propham could be used as an effective herbicide against young grasses when applied to the soil, Allard et al. (1) believed its greatest potential was as a general herbicide when mixed with phenoxy herbicides or certain other herbicides.

The action of propham is greatly inhibited by the presence of organic matter in the soil (39). Even moisture inactivates propham (30). Because organic matter and moisture inactivate propham it is not effective throughout an entire growing season; thus, its use is limited.

Ennis (15), in each of the 13 monocots tested, noted lack of root and shoot elongation with concurrent swelling of these parts. Roots appeared to be stubby and bulbous, and the coleoptile region was markedly swollen.

Microscopic examination of roots and shoots showed abnormal cytological behavior namely: interrupted mitotic cycle, blocked metaphases, multinucleate cells, giant nuclei, high polyploidy, chromatid fragmentation, multipolar spindles, and chromosome bridges (13, 15, 16).

Mann and Storey (38, 46) reported that chromosomes in propham treated tissue shortened at the rate of 50% in three hours; however, chromosome configuration differed from those created by colchicine by chromatids being less spread. Chromosome separation also occurs with the shortening but after about five hours in treatments above 50 parts per million (ppm) of propham chromosomes begin to disintegrate and granulate; thus, making dividing cells indistinguishable from cells in a metabolic stage (46).

MATERIALS AND METHODS

Treatments

Five treatments compared with control material were: 1. 6% DMSO; 2. 10 ppm propham; 3. 100 ppm propham; 4. 10 ppm propham in 6% DMSO; and 5. 100 ppm propham in 6% DMSO. The 6% concentration of DMSO was used because it greatly inhibited root growth but did not kill the seedlings. The concentrations of propham used were suggested by Ennis (14), who found that 0.05 ppm to 300 ppm propham inhibited rye root tip mitosis.

The above chemicals were freshly prepared not more than two hours before use.

Preparation of Chemicals

Technical grade propham (0.10g) was dissolved in a 1000 ml flask containing 4 ml of absolute ethyl alcohol. The mixture was shaken for five minutes. Approximately 750 ml of distilled water was then added with stirring. The mixture was stirred for two or three minutes to prevent precipitation. The solution was brought to volume in a 1000 ml graduated cylinder, resulting in a concentration of 100 ppm propham. To form a 10 ppm propham solution, 100 ml of the stock solution was added to 896.4 ml of distilled water and 3.6 ml of absolute ethyl alcohol and mixed thoroughly.

A second propham solution was prepared by dissolving 0.106 g of herbicide in 996 ml of distilled water and 4 ml of absolute ethyl alcohol. Of this stock solution, 470 ml was added to 30 ml of DMSO and 0.2 ml of absolute ethyl alcohol resulting in a 100 ppm propham in 6% DMSO solution. A 10 ppm propham in 6% DMSO solution was prepared by mixing 30 ml of DMSO,

0.2 ml of absolute ethyl alcohol, and 100 ml of the stock solution. A 6% DMSO solution was prepared by mixing 468 ml distilled water, 30 ml DMSO, and 2 ml of absolute ethyl alcohol. The control was prepared by mixing 498 ml distilled water with 2 ml of absolute ethyl alcohol.

Plant Preparation and Treatment

Seeds of Secale cereale L. were germinated in 28 cm x 18 cm x 4.5 cm pyrex cake dishes on tissues soaked in distilled water. Dishes were loosely covered with cellophane to increase humidity and allow ventilation. When the roots of approximately 70% of the seedlings were 2.5 cm in length, healthy plants were transferred to pyrex cake dishes containing tissues soaked with the test solution. Two replications were used. A minimum of 75 seedlings were placed in each cake dish. Cellophane was placed loosely over the dishes. Root tips from at least 10, but not more than 12 seedlings per treatment and replication were excised at 1, 3, 6, 12, 24, and 48 hours folfowing treatment.

Preparation of Material

Excised root tips were immediately placed in individual containers of Craf or Newcomers (36) killing and fixing solution. Craf fixed root tips were prepared by the paraffin method (40). Root tips fixed in Newcomers solution were observed employing the aceto-carmine squash technique.

Craf-fixed root tips were stained with erythrosin for ease of orientation when embedding in paraffin. Paraffin blocks were cut in 12 micron sections on a rotary microtome. Sections were mounted on slides using gelatin adhesive and then stained with crystal violet and orange G. Slides were sealed with piccolite resin.

Serial sections of root tips were made and observed, however, mitotic frequency was determined from median longitudinal sections, (one per root tip), from a minimum of nine and a maximum of 15 root tips per time treatment. Anatomical and chromosomal abnormalities were also observed in serial sections.

Root tips killed and fixed in Newcomers solution were soaked in a 1:1 solution of hydrochloric acid and 95% ethyl alcohol for 1.5 minutes.

Using the squash preparation method, a minimum of 50 plates, where possible, were interpreted per time treatment (25 in each replication).

Abnormalities observed in both the paraffin sections and aceto-carmine squash preparations were documented with photomicrographs.

RESULTS AND DISCUSSION

Mitotic Abnormalities in Sectional Root Tips

Median longitudinal sections of paraffin embedded root tips were used to determine mitotic frequency (Table 1) and support squash preparation findings. Division frequencies were consistent within treatments and controls.

DMSO readily inhibited mitosis. One hour after treatment, mitosis had greatly decreased. Because of this reduction in the number of divisions, roots grew very slowly as compared with control. The presence of propham during the first six hours increased mitotic frequency compared to the control. From 6 to 48 hours division frequency remained higher in the seedlings treated with the 100 ppm concentrations of propham as compared to those receiving the 10 ppm concentration; however, both were considerably less than controls. This may be attributed in part to propham's ability to granulate chromosomes and cause clumping, making dividing cells difficult to distinguish from those in metabolic stage. At 48 hours propham treated root tips (Figure 1) show mitotic activity confined to a narrow region between xylem and root cap as contrasted to the normal condition in the control (Figure 2). Cell size also increased considerably in propham treated roots.

When propham and DMSO were combined, mitotic frequency was considerably reduced in both 10 ppm and 100 ppm concentrations of propham. Mitotic frequency was similar in both concentrations. An additive effect was thus observed as determined by a comparison of percentage of abnormal cells and may be caused in part by increased speed of penetration.

Table 1. Effect of propham and DMSO on mitotic cell division in rye root tips.

Treatment	Average number of mitotic divisions per median longitudinal section[a]					
	Hours					
	1	3	6	12	24	48
Control	32.3	20.0	22.6	22.0	29.3	13.9
6% DMSO	20.9	5.4	2.6	1.4	1.7	4.1
10 ppm propham	42.4	37.9	28.8	15.1	7.6	2.1
100 ppm propham	44.5	34.2	39.8	19.8	13.1	4.9
10 ppm propham 6% DMSO	15.6	1.9	1.5	1.5	4.3	0.3
100 ppm propham 6% DMSO	15.4	2.4	0.1	0.1	0.4	1.7

[a]One median longitudinal section was examined per root tip. The number of root tips per time treatment ranged from 9 to 15.

All abnormalities later discussed in squash preparation analysis were observed in serial sections.

Mitotic Abnormalities in Aceto-Carmine Squash Preparations

The summary of abnormalities in root tips following treatment with DMSO and stained with aceto-carmine appears in table 2. No abnormalities were observed in control material. In all treatments the number of prophase and metaphase plates were more numberous than the anaphase and telophase plates. This is partially due to the presence of blocked metaphases in all treatments. The abnormalities noted in DMSO treated root tips were arrested metaphases, shortened chromosomes, multipolar spindles (Figure 3), stickiness (Figure 4), and mitotic inhibition.

DMSO, at 6%, almost completely inhibited mitotic division at 6, 12, 24, and 48 hours. The high frequency of metaphase plates compared to anaphase plates is due to arrested metaphases. The chromosome configurations in DMSO arrested metaphases closely resemble those induced by propham. However, chromatid separation is not so distinct in DMSO or propham treated plants as in colchicine treated material. The chromosomes from plants treated with DMSO were shorter than control chromosomes.

The cause of the multipolar condition is not known. If a multipolar cell is allowed to complete division it results in a corresponding number of nuclei and cells varying in amounts of chromosomal material. Stickiness, caused by DMSO, varied in severity but in some cases (Figure 4), would result in abnormal mitosis. A pilot study on the effect of DMSO on rye pollen mother cells showed extreme stickiness (Figure 5), and a number of microspores containing 14 chromosomes (Figure 6).

Table 2. Effect of 6% DMSO on frequency of abnormalities[a] in rye root tip mitoses in squash preparations.

Treatment	Hours	Stage of cell division: Prophase and Metaphase — Number of div. observed	Prophase and Metaphase — Number of div. abnormal	Anaphase and Telophase — Number of div. observed	Anaphase and Telophase — Number of div. abnormal	Total number of cell div. exam.	Total number abnormal cell div.	Total percent abnormal cell div.
Control	1	36	0	86	0	122	0	0
	3	13	0	45	0	58	0	0
	6	19	0	43	0	62	0	0
	12	22	0	47	0	69	0	0
	24	21	0	70	0	91	0	0
	48	16	0	52	0	68	0	0
6% DMSO	1	54	40	32	14	86	54	62.8
	3	7	5	7	7	14	12	85.7
	6	0	0	0	0	3	3	100.0
	12	0	0	0	0	4	4	100.0
	24	0	0	0	0	3	3	100.0
	48	0	0	0	0	4	4	100.0

[a]Abnormalities include shortened chromosomes, multipolar spindles, stickiness, arrested metaphases, and multinucleate cells.

The 10 ppm and 100 ppm propham treatments are compared in table 3. In both concentrations the types of abnormalities were the same and included blocked metaphases, multipolar spindles, polyploidy, binucleate cells (Figure 8), chromosome shortening (Figure 7), stickiness (Figure 9), and chromosome granulation. In the 10 ppm propham treatment the multipolar condition was considerably more frequent than in the higher concentration. The frequency of multipolar cells increased from 12 to 24 hours and then rapidly decreased to zero at 48 hours. The number of poles present increased with time up to and including 24 hours. As many as four poles were sometimes present at 24 hours.

The frequency of polyploidy was higher in the 100 ppm concentration of propham than in the 10 ppm concentration. The presence of multinucleate cells may be attributed to multipolar spindles.

Blocked metaphases and shortened chromosomes were very common in both concentrations of propham. The increased number of prophase and metaphase plates compared to anaphase and telophase plates resulted from blocked metaphases. After six hours all chromosomes regardless of stage were considerably shorter than controls. Chromosome granulation began as early as three hours after treatment. Not all chromosomes became granular, however, even 48 hours after treatment.

Table 4 shows results observed from root tip squash preparation stained with aceto-carmine in which 10 ppm propham and 6% DMSO are compared with 100 ppm propham and 6% DMSO. Abnormalities found included multipolar anaphases (Figure 10), stickiness, multinucleate cells, shortened chromosomes, chromosome granulation, blocked metaphases, and severe mitotic inhibition. The multipolar condition occurred frequently in 10 ppm propham and 6% DMSO but rarely in the 100 ppm propham and 6% DMSO treatment. No stickiness was

Table 3. Effect of 10 ppm and 100 ppm propham on the production of abnormalities[a] in mitosis in squash preparations of rye root tips.

Treatment	Hours	Stage of cell division: Prophase and Metaphase — Number of div. observed	Prophase and Metaphase — Number of div. abnormal	Anaphase and Telophase — Number of div. observed	Anaphase and Telophase — Number of div. abnormal	Total number of cell div. exam.	Total number abnormal cell div.	Total percent abnormal cell div.
10 ppm propham	1	56	55	12	4	68	59	86.7
	3	66	64	3	3	69	67	97.1
	6	110	110	22	22	132	132	100.0
	12	77	77	7	7	83	83	100.0
	24	53	53	6	6	59	59	100.0
	48	15	15	0	0	15	15	100.0
100 ppm propham	1	77	76	2	2	79	78	99.0
	3	56	56	4	2	60	58	96.7
	6	61	61	0	0	61	61	100.0
	12	55	55	2	2	57	57	100.0
	24	65	65	0	0	65	65	100.0
	48	25	25	0	0	25	25	100.0

[a]Abnormalities include polyploid cells, multipolar spindles, blocked metaphases, multinucleate cells, shortened chromosomes, and stickiness.

Table 4. Effect of 10 ppm and 100 ppm propham with 6% DMSO on the production of abnormalities[a] in mitosis in squash preparations of rye root tips.

Treatment	Hours	Stage of cell division: Prophase and Metaphase — Number of div. observed	Prophase and Metaphase — Number of div. abnormal	Anaphase and Telophase — Number of div. observed	Anaphase and Telophase — Number of div. abnormal	Total number of cell div. exam.	Total number abnormal cell div.	Total percent abnormal cell div.
	1	37	37	21	21	58	58	100.0
	3	5	5	5	5	10	10	100.0
10 ppm	6	0	0	0	0	4	4	100.0
propham	12	0	0	0	0	3	3	100.0
6% DMSO	24	0	0	0	0	3	3	100.0
	48	0	0	0	0	3	3	100.0
	1	59	59	1	1	60	60	100.0
	3	7	7	5	5	12	12	100.0
100 ppm	6	0	0	0	0	5	5	100.0
propham	12	0	0	0	0	3	3	100.0
6% DMSO	24	0	0	0	0	3	3	100.0
	48	0	0	0	0	4	4	100.0

[a]Abnormalities include multipolar spindles, blocked metaphases, multinucleate cells, shortened chromosomes, and stickiness.

observed in the 100 ppm propham and 6% DMSO treatments. Blocked metaphases were more common in 100 ppm propham and 6% DMSO than in 10 ppm propham and 6% DMSO. No polyploidy was observed in either treatment.

A comparison of table 4 with tables 2 and 3 indicate that an additive detrimental effect was obtained by combining propham and DMSO. Polyploidy was the only abnormality not observed in the propham DMSO combination that was observed with propham alone.

CONCLUSIONS

1. DMSO at 6% concentration shows a marked effect on rye root tip mitosis. Abnormalities observed include stickiness, arrested metaphases, chromosome shortening, multipolar spindles, multinucleate cells, and mitotic inhibition. These abnormalities could be observed one hour after treatment.
2. Rye root tips treated with propham at 10 ppm or 100 ppm concentrations resulted in a number of abnormalities: polyploidy, arrested metaphases, stickiness, multipolar anaphases, shortened chromosomes, multinucleate cells, chromosome granulation, and a stimulatory effect noted by the increase in mitosis for the first six hours. The multipolar condition was more frequent in the 10 ppm concentration. Polyploidy, however, occurred more frequently in the 100 ppm concentration of propham.
3. When 6% DMSO was combined with 10 ppm and 100 ppm propham an additive detrimental effect was observed in rye root tip mitosis. Abnormalities include multipolar anaphases, arrested metaphases, shortened chromosomes, stickiness, multinucleate cells, chromosome granulation, and severe mitotic inhibition.
4. DMSO's penetrating ability and its ability to adversely effect mitosis demonstrates its potential as a carrier of herbicides and as an agriculture chemical.

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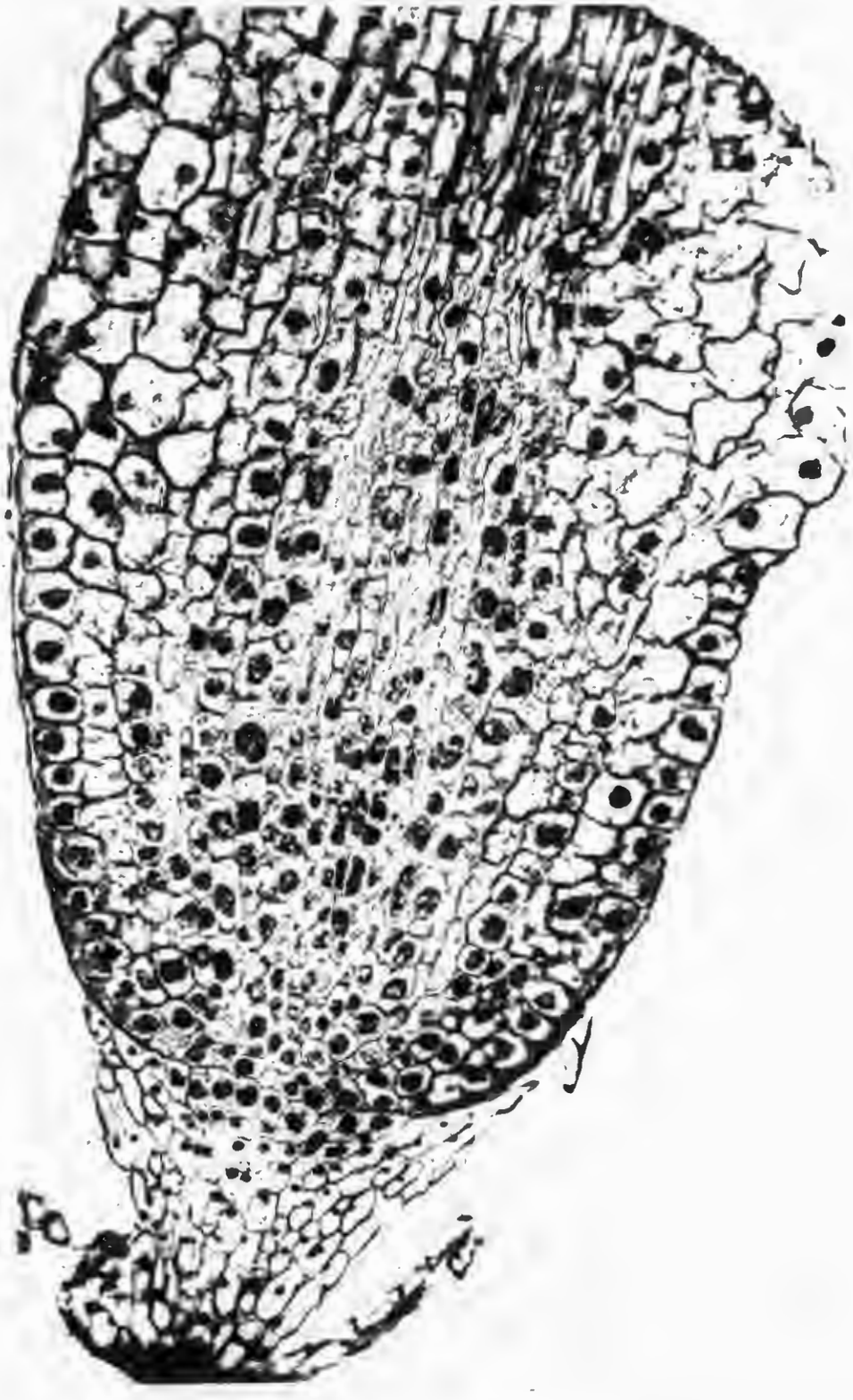

Figure 1. Rye root tip treated with 100 ppm propham for 48 hours. Note small region of mitotic activity and large cell size. 183X

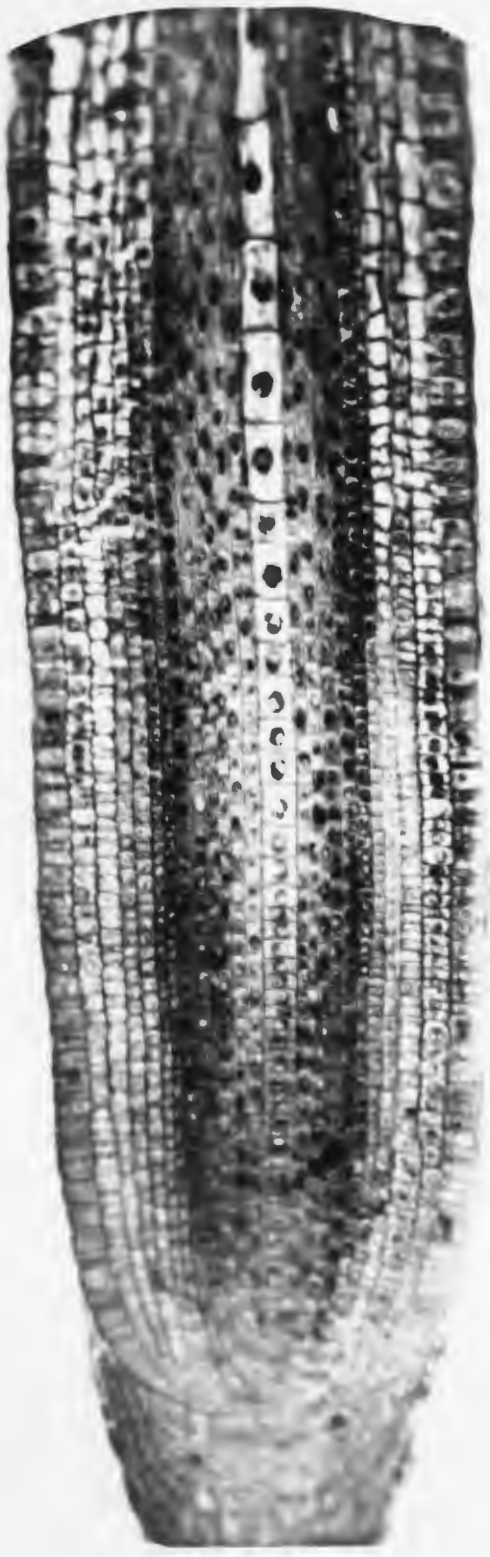

Figure 2. Normal development of rye root tip in control. 180X

Figure 3. Tripolar division in a rye root tip cell following a one hour application of 6% DMSO. 5786X

Figure 4. Chromosome stickiness in rye root tip cell resulting from a one hour application of 6% DMSO. 5477X

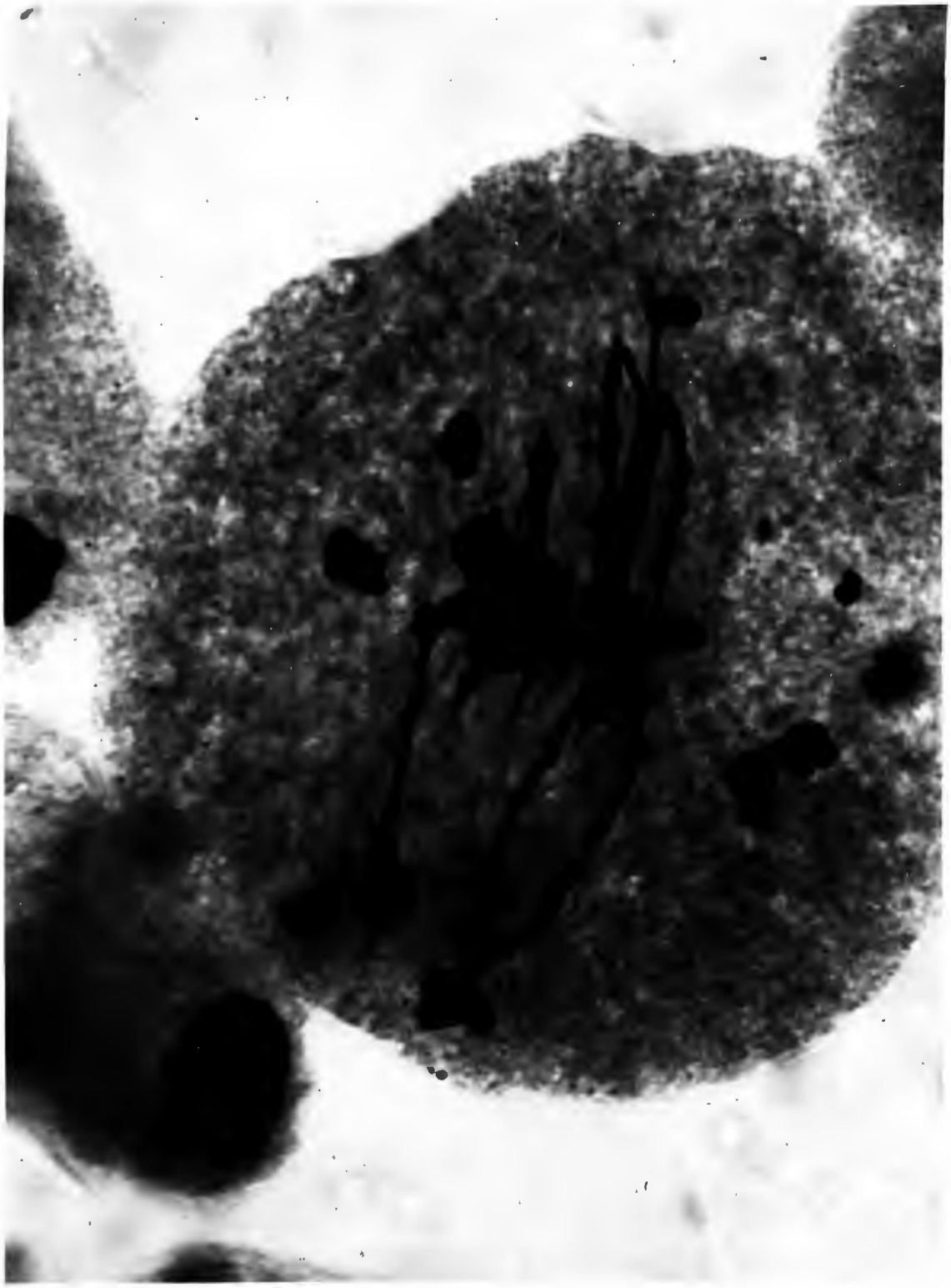

Figure 5. Abnormal telophase I in a pollen mother cell division showing extreme stickiness and lagging dyads in plant grown from a seed soaked in 19% DMSO for 24 hours. 1498X

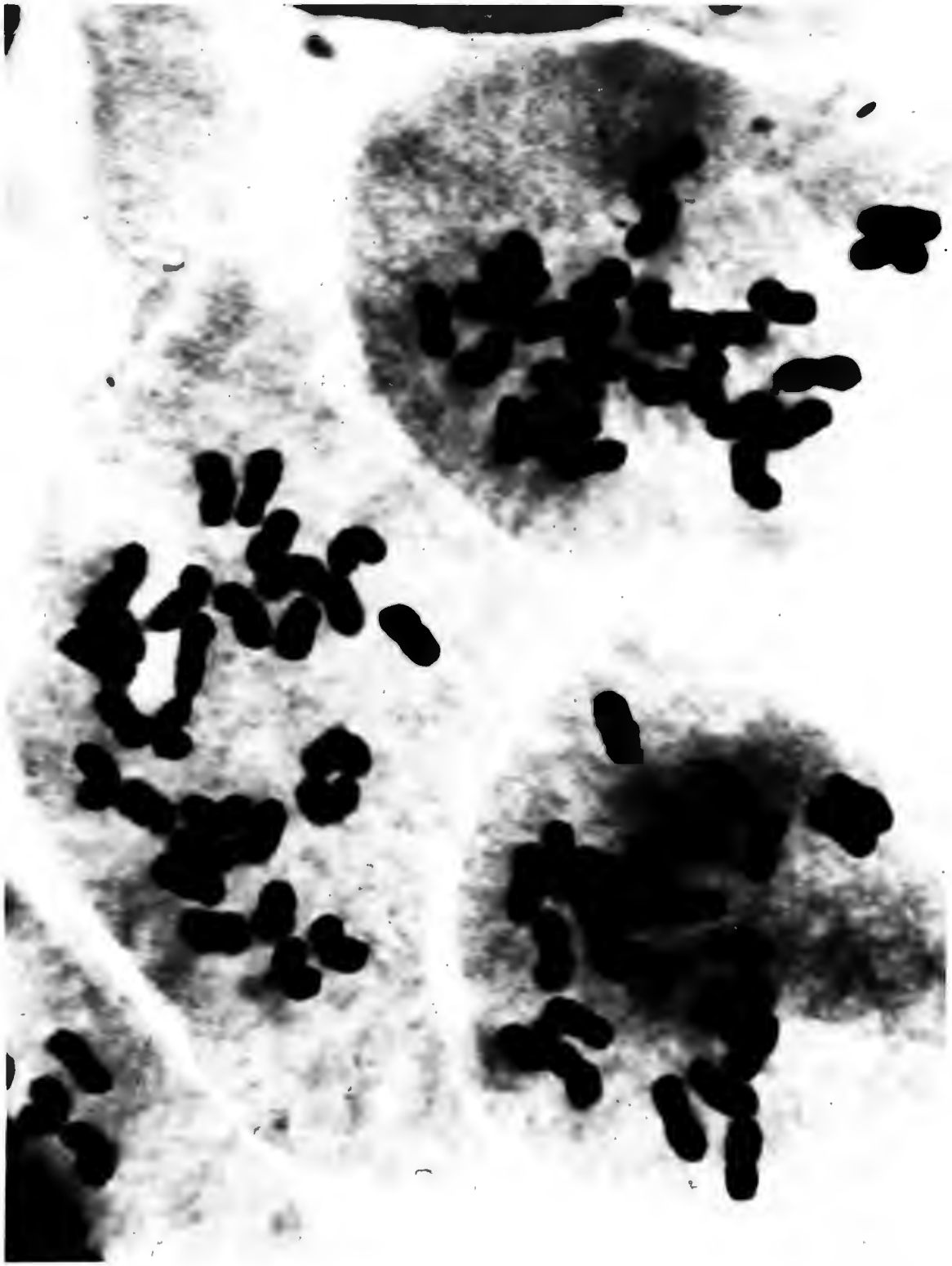

Figure 6. Abnormal anaphase II in three pollen mother cell divisions following a two minute treatment of 25% DMSO to the spike in the boot stage. 2010X

Figure 7. Chromosome condensation induced by a 24 hour application of 10 ppm propham to root tip. 3946X

Figure 8. Binucleate cell of rye root tip resulting from a one hour treatment of 6% DMSO and 100 ppm propham solution. 3472X

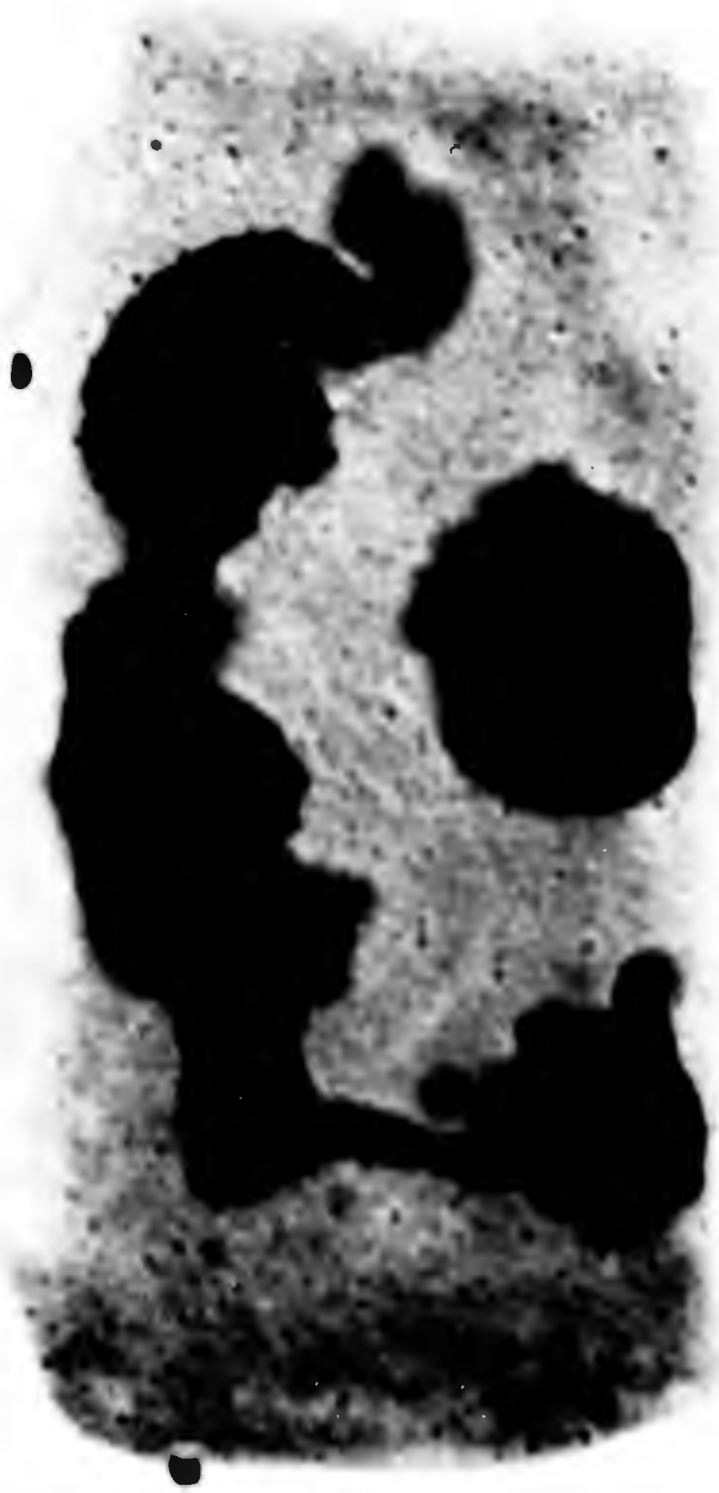

Figure 9. Abnormal telophase division in a rye root tip cell showing stickiness and chromosome clumping induced by a six hour application of 10 ppm propham. 5009X

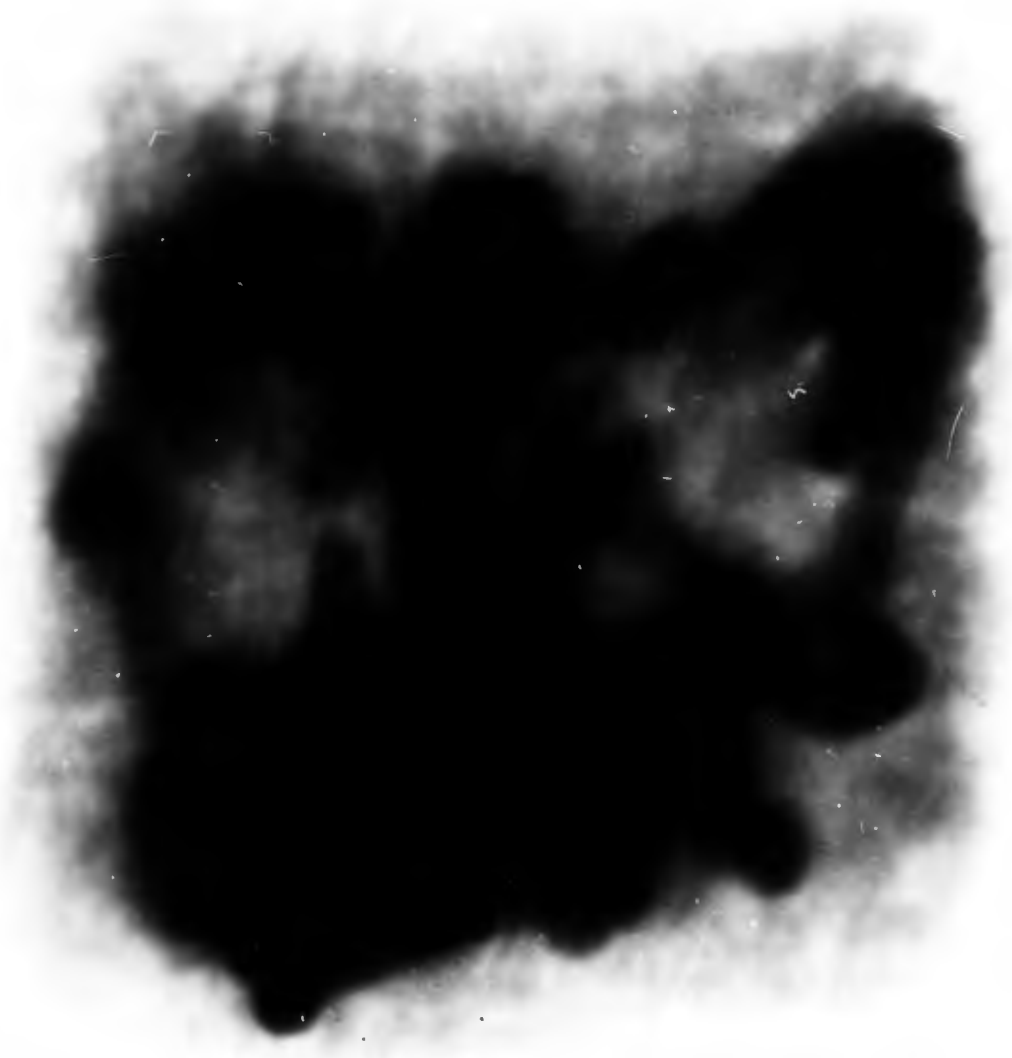

Figure 10. Tripolar division in a rye root tip cell induced by one hour application of 6% DMSO and 10 ppm propham. 4427X